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Acid phosphatase activity in certain tissues of the fresh water fish *Notopterus notopterus* exposed to Quinalphos.

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Abstract: Present study investigates the acid phosphatase activity in certain tissues of the fresh water fish *Notopterus notopterus* in control, 15, 30, 45, 60 days exposure to different fractions of 96 hr LC_{50} of Quinalphos in liver, kidney and brain. Maximum inhibition was observed at $1/5^{th}$ fraction at 60 days and minimum at $1/20^{th}$ fraction after 15 days exposure. Maximum stimulation was observed at $1/25^{th}$ fraction after 45 days exposure and minimum after 45 days at $1/25^{th}$ fraction.

***Index Terms* - Acid phosphatase, *Notopterus notopterus*, Quinalphos.**

I. Introduction

The most toxic substances are the pesticides released into the environment. While they have greatly added to human well-being,they have major harmful impacts on non-target species. The direct application of pesticides, spray drift, airborne spray, deforestation and drainage from farm land hit the marine environment. Pollution of the water supply not only depends on the quantity and characteristics of pesticides, but also on the biology and composition of the water system. The use of pesticides in India is about 3% of overall global use and rises by 2 to 5% annually. [1] The Quinalphos (Curacron) 50% EC has been selected for the present investigation on account of its broad-spectrum killing power. It is a persistent pesticide used for the control of soil insects, pests of vegetables, fruit and field crops, also as a residual spray for dairy and livestock pests.

Fish are repeatedly compelled to find themselves in polluted environments and can be used as a research organism because they are the most understandable aquatic organisms and markers of potential environmental toxins such as pesticides are available onthe frontlines [2]. The bioaccumulations of such pesticides endanger the long-term survival of fish by disturbing the biological ties between species and biodiversity losses [3][4]. Pesticides long-term exposure causes metabolic disturbance, behavioral changes, histopathological injury, haematological changes, biochemical changes, immune-suppression, degradation of hormones, reduced intelligence, and cancer abnormalities [5][6][7][8][9][10][11].

II. RESEARCH METHODOLOGY

The test fish *Notopterus notopterus* were collected from Bisalpur Dam District Tonk Rajasthan by fisherman. Fish acclimatized to the laboratory condition after wash with 1% $Kmno_4$ solution. The technical grade of quinalphos was used for the experiment. The quinalphos stalk solution of 1.0 gm/L in distilled water was prepared and desired concentrations were obtained by using the standard method [12].

10 fishes were transferred in each concentration ($1/5^{th}$, $1/10^{th}$, $1/15^{th}$, $1/20^{th}$ and $1/25^{th}$ fractions of 96 hr LC_{50}). During acclimatization fish were fed twice a day with rice bran oil cake and chilled crustacean including daphnia and Cyclops to avoid the starvation effect on the experiment [13]. Solutions were changed after each 24 hr to avoid the contamination by remaining food particles and animals excretory product. Liver, kidney and brain were taken out from the dead fish and transferred these organs in ice cold petridishes containing 0.25 M sucrose solution tissue homogenates (5%) were prepared separately using 0.25 M sucrose solution with a potter Elvehjem homogenizer. Homogenates were centrifuged at 900 g under cold conditions (5.0±1.0 ^{0}C) and supernatants were used for enzyme study. Acid phosphatase activity was measured by the methods of Shinowara et al. [14][15]. Statistical significance of the difference between the control and experimental values was calculated by students't' test [16][17].

III. RESULTS AND DISCUSSION

The acid phosphatase activity in control as well as in quinalphos exposed fishes along with the percentage alterations in liver, kidney and brain of Notopterus notopterus are given in table 1-2. From these tables following important observations can be made:

Hepatic acid phosphatase inhibited significantly ($P<0.05$, $P<0.01$ and $P<0.001$) stimulation in 15, 30, 45 and 60 days exposed fishes. Maximum inhibition was observed at $1/5^{th}$ fraction at 60 days and minimum at $1/20^{th}$ fraction after 30 days exposure. In kidney, activity of acid phosphatase inhibited significantly ($P<0.05$) at all fractions except $1/5^{th}$,$1/10^{th}$ and $1/15^{th}$ fraction after 60 days exposure where significant ($P<0.05$) inhibition was observed. In $1/25^{th}$ fractions acid phosphatase stimulated insignificantly in 15, 30, 45 and 60 days exposed fishes. Maximum inhibition was observed at $1/5^{th}$ fraction in 60 days exposed fishes. Maximum stimulation was observed at $1/25^{th}$ fraction after 15 days and minimum at $1/25^{th}$ fraction after 60 days exposure. In brain, acid phosphatase inhibited insignificantly at $1/5^{th}$,$1/10^{th}$,$1/15^{th}$,$1/20^{th}$ and $1/25^{th}$ fractions after 15 and 30 days and significant after 45 and 60 days. Insignificant stimulation was observed after 15 and 30 days while significant stimulation ($P<0.05$) was observed after 45 and 60 days exposure.

Maximum inhibition was observed at $1/5^{th}$ fraction after 60 days and minimum at $1/20^{th}$ fraction after 15 days. Maximum stimulation was observed at $1/25^{th}$ fraction after 45 days and minimum after 45 days at $1/25^{th}$ fraction.

Table – 1 Acid phosphatase activity in certain tissues of *Notopterus notopterus* in control, 15 and 30 days exposure to different fractions of 96 hr LC_{50} of Quinalphos.

Fraction of 96 hrs LC_{50}	Specific activity : Mg of pi liberated/Mg protein/hr.					
	Liver	% alter	Kideny	% alter	Brain	% alter
15 Days exposure						
Control	68.26 ± 2.65	-	3.95 ± 0.65	-	1.60 ± 0.25	-
$1/5^{th}$	40.10 ± 2.50	41.25**	3.08 ± 0.55	22.03	0.88 ± 0.22	45
$1/10^{th}$	43.92 ± 2.47	35.66**	3.24 ± 0.45	17.98	0.95 ± 0.15	40.63
$1/15^{th}$	46.45 ± 1.65	31.95**	3.26 ± 0.68	7.59	1.08 ± 0.25	32.5
$1/20^{th}$	65.35 ± 1.90	17.72**	3.89 ± 0.70	16.2	1.25 ±0.27	21.88
$1/25^{th}$	87.60 ± 2.30	28.34**	4.79 ± 0.45	21.27	2.05 ± 0.25	21.88
30 Days exposure						
Control	68.27 ± 2.52	-	3.94 ± 0.40	-	1.62 ± 0.24	-
$1/5^{th}$	34.90 ± 2.32	48.88***	2.72 ± 0.20	30.97	0.80 ± 0.21	50.62
$1/10^{th}$	55.98 ± 2.10	47.29***	2.87 ± 0.21	27.65	0.92 ± 0.22	43.21
$1/15^{th}$	38.72 ± 1.92	43.29***	3.05 ± 0.25	22.59	1.02 ± 0.26	37.04
$1/20^{th}$	42.45 ± 2.60	37.82***	3.28 ± 0.24	16.75	1.15 ± 0.24	29.02
$1/25^{th}$	85.30 ± 1.85	26.32**	4.45 ± 0.45	22.95	2.09 ± 0.27	22.84

All the values are mean ± S.E. of three observations

Values are significant at *P <0.05, **P <0.01, ***P <0.001

Table – 2 Acid phosphatase activity in certain tissues of *Notopterus notopterus* in control, 45 and 60 days exposure to different fractions of 96 hr LC_{50} of Quinalphos.

Fraction of 96 hrs LC_{50}	Specific activity : Mg of pi liberated/Mg protein/hr.					
	Liver	% alter	Kideny	% alter	Brain	% alter
45 Days exposure						
Control	68.24 ± 2.60	-	3.94 ± 0.70	-	1.58 ± 0.15	-
$1/5^{th}$	28.72 ± 2.40	57.92**	2.49 ± 0.35	36.81	0.68 ± 0.14	59.96*
$1/10^{th}$	33.65 ± 2.30	50.69***	2.65 ± 0.61	32.74	0.77 ± 0.15	51.27*
$1/15^{th}$	36.65 ± 2.30	46.63***	2.69 ± 0.85	24.87	0.92 ± 0.17	41.77*
$1/20^{th}$	64.42 ± 2.71	41.61**	3.14 ± 0.85	20.31	0.98 ± 0.14	37.98*
$1/25^{th}$	85.55 ± 1.98	33.25**	4.45 ± 0.72	12.44	2.27 ± 0.18	25.95
60 Days exposure						

Control	68.24 ± 2.59	-	3.92 ± 0.31	-	1.57 ± 0.13	-
1/5th	22.80 ± 1.82	66.73**	2.25 ± 0.25	42.61*	0.60 ± 0.17	61.78*
1/10th	29.05 ± 0.41	57.43***	2.51 ± 0.24	35.97*	0.72 ± 0.14	54.14*
1/15th	33.28 ± 2.61	51.23***	2.80 ± 0.23	28.57*	0.85 ± 0.15	45.86*
1/20th	55.95 ± 2.32	47.32***	3.08 ± 0.20	21.43*	0.95 ± 0.12	39.49*
1/25th	81.71 ± 2.17	24.23**	4.25 ± 0.24	17.09*	2.14 ± 0.11	29.39

All the values are mean ± S.E. of three observations

Values are significant at *P <0.05, **P <0.01, ***P <0.001

A number of alterations were observed in the enzymes of various organs of *Notopterus notopters* when exposed to different sublethal concentrations of quinalphos. It was found that different enzymes respond differently. However, in most cases alteration were observed to be concentrations and duration dependent. Inhibition in acid phosphatase activity observed in this investigation after sublethal concentrations of quinalphos. It might be due to the result of disintegration of the affected cells or direct binding of the metal ions with enzymes protein. Loss in acid phosphatases activity in different tissues indicates lysosomal damage since it is one of the most characteristics hydrolase of the lysosome. Secondly some metals like lead are known to form inclusion bodies which are never found in normal cells, but are produced after lysosomal defect or after the combination of lead with lysosome [18]. These inclusion bodies caused cell injury by altering lysosomal structure functions as evidence by reduced acid phosphatase activity. Author assumes that this carbonic compound may also induce several lysosomal defects, like the formation of inclusion bodies.

Jeckim et al. observed the marked reduction in the hepatic acid and alkaline phosphatases in killifish Fundulus heteroclitus following exposure to copper and mercury [19]. Hinton *et al.* [20] and Kendall [21]and Kendall and Hawkins [22]studied the effect of methyl mercuric chloride and observed marked inhibitory inhibition in acid and alkaline phosphatase activity in liver and kidney of channel cat fish *Ictalurus punctatus*.

However, auther of this investigation, assumed that all these interactions and processes held simultaneously when fish were exposed to quinalphosand finally affect the activity of phosphatase but the actual mechanism of quinalphos effect on Acid phosphatase is not well known.

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